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Clinical evaluation of corneal epithelialization after photorefractive keratectomy in patients treated with polydeoxyribonucleotide (PDRN) eye drops: A randomized, double-blind, placebo-controlled trial

M. LAZZAROTTO, E.M. TOMASELLO, A. CAPOROSSI

Department of Ophthalmology and Neurosurgery, University of Siena, Siena - Italy

PURPOSE. *The effect of polydeoxyribonucleotide (PDRN) eye drops vs placebo on corneal epithelial healing after photorefractive keratectomy (PRK) for correction of myopic and myopic-astigmatic defects was evaluated in a randomized, double-blind clinical trial. Primary endpoint for efficacy was the evolution of corneal re-epithelialization. Secondary endpoint was the evaluation of PDRN eye drops tolerability.*

METHODS. *Sixty eyes were enrolled in the study, randomly allocated into standard therapy plus placebo eye drops (30 eyes), or standard therapy plus PDRN eye drops (30 eyes). Checks were carried out preoperatively and at days 1, 2, 3, and 7 of the follow-up. Six eyes dropped out (four in PDRN group, two in placebo group) for reasons unrelated to the study.*

RESULTS. *On day 2, the disepithelialized area was 8.4 mm^2 ± 9.2 (mean ± SD) in controls and 6.0 mm^2 ± 6.8 in PDRN group. On day 3 a complete corneal re-epithelialization was found in 20 out of 26 (77%) eyes of PDRN group and in 17 out of 28 (61%) eyes of placebo group ($p<0.05$ in percentage terms). On day 7 of follow-up, all eyes appeared to be completely re-epithelialized. The mean score of corneal evaluation on day 3 was 2.9 in PDRN group and 3.75 in control group ($p<0.05$ between groups). No adverse events occurred during the study.*

CONCLUSIONS. *The data of the study have shown that after PRK, PDRN stimulates corneal epithelium regeneration. PDRN eye drops administration four times a day is well tolerated by patients during the re-epithelialization stage. A much larger clinical study should be performed in order to prove the results obtained in this pilot study.(Eur J Ophthalmol 2004; 14: 284-9)*

KEY WORDS. *Cornea, Epithelialization, Polydeoxyribonucleotide, PDRN, Photorefractive keratectomy, Wound healing*

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INTRODUCTION

Polydeoxyribonucleotide (PDRN) eye drops (Placentex eye drops, 7.5 mg/10 ml, Mastelli Srl-Sanremo, Italy) consist of low molecular weight DNA fractions that can be defined as deoxyribonucleotide linear polymers. Purine and pyrimidine nucleotides are the monomeric units that are combined by phosphodiester bonds. The compound is obtained by extraction from trout sperm and is then purified and sterilized to obtain over 95% pure active principals without pharmacologically active proteins and peptides (Registration Dossier, Ministry of Health). PDRN eye drops consist of long chains of nucleotides (from 50 to 2000 pairs of bases), different from a similar product (Vitacic eye drops, Novartis Pharma), commercialized in France and other European countries, which consists of single purine and pyrimidine nucleotides.

The preparation has been used for years in therapy as a tissue repair-stimulating agent in diseases such as wounds and burns. The PDRN healing effect is attributed to the nucleotides by which it is formed. Wang et al were probably the first authors to establish a link between nucleotides and wound healing. They demonstrated the stimulating effect of extracellular ATP and ADP on cell multiplication, on DNA synthesis, and on wound healing (1). Nucleic acids of damaged cells, released in the extracellular environment, are rapidly lysed into nucleotides that trigger a repair response and are able to activate various cell types (2-5). Other studies have shown that the action of nucleotides is mediated by the stimulation of type A2 purinergic receptors (6-9). A second mechanism of action for nucleotides to physiologically increase cell regeneration is to stimulate the synthesis of new nucleic acids necessary for cell multiplication. Preformed nucleotides in fact activate the salvage metabolic pathways (recovery of preformed nucleotides for the synthesis of nucleic acids) as an alternative to the traditional metabolic pathways of the *ex novo* synthesis (10, 11) (nucleotide neo-synthesis starting from amino acids with a high energy consumption). Salvage pathways favor a quicker synthesis rate and a lower energy consumption (12).

In vitro studies have shown that PDRN can stimulate the growth of human fibroblast primary cultures at concentrations of 20 to 100 mg/ml and that cell proliferation is at least partly mediated by stimulating type A2 purinergic receptors (13, 14) and by activating "salvage pathways."

Salvage pathways and nucleotide sugar phosphate are important corneal energy saving mechanisms (15, 16).

In our previous pilot study (unpublished data), it was observed that the administration of PDRN eye drops to subjects after photorefractive keratectomy (PRK) caused an increase in the re-epithelialization rate of the corneal lesion. Therefore, further investigations have been planned to evaluate with statistical significance the stimulating effect of PDRN eye drops on the regeneration of the corneal lens epithelium in subjects after PRK.

PATIENTS AND METHODS

We enrolled 60 eyes (Tab. I) of 46 patients of both sexes who were candidates for PRK. Five patients (six eyes, two of the control group and four of the PDRN group) dropped out of the study immediately after surgery for reasons unrelated to the study. A total of 41 patients (mean age 33.4 ± 9.5 years) completed the investigation according to the experimental protocol for a total of 54 examined eyes, 26 treated with PDRN and 28 with placebo (Tab. II). Out of the 14 patients operated on both sides, 5 received PDRN on one eye and placebo on the other. The other 9 patients were treated on both eyes either with PDRN alone (4 patients) or with placebo alone (5 patients).

After local anesthesia with oxybuprocaine eye drops a pupil centrated circular area was marked by using a 10 mm epithelial marker (Asico). Epithelial removal was started at the periphery of the cornea and ended in its center by a blunt spatula (Vinciguerra's spatula, Asico). Laser ablation (17) was performed by a Technolas 217c B&L excimer laser and Planoscan 2000 software. Before surgery, patients took a 10 mg ketorolac tablet. If needed, treatment continued postoperatively up to a maximum of three tablets a day. After surgery the eyes were medicated with cyclopentolate, diclofenac, and tobramycin (eye drops). A therapeutic soft contact lens (H55, Schalcon) was fitted and left *in situ* until complete re-epithelialization. For all eyes we used the same type of lens, made by Methafilcon A (55%) and water (45%), with a 14.2 mm diameter and a curvature radius of 8.8 mm. The

TABLE I - RANDOMIZED SUBJECTS

Subjects	Total	PDRN	Placebo
Enrolled eyes	60	30	30
Enrolled patients	46	26	25
Examined eyes	54	26	28
Examined patients	41	23	23
Drop-out eyes	6	4	2
Drop-out patients	5	3	2

Both eyes of 14 patients were enrolled: five patients received polydeoxyribonucleotide (PDRN) on one eye and placebo on the other, four patients received PDRN on both eyes, and five patients received placebo on both eyes

TABLE II - COMPOSITION OF POLYDEOXYRIBONUCLEOTIDE (PDRN) AND PLACEBO EYE DROPS ADMINISTERED IN THE STUDY

Components	PDRN	Placebo
Polydeoxyribonucleotide	7.5	—
Excipients		
Polyvinylpyrrolidone	750	750
Monobasic phosphate sodium	16	16
Bibasic phosphate sodium	100	100
Sodium edetate	1	1
Methyl-p-hydroxybenzoate	9	9
Propyl-p-hydroxybenzoate	3	3
Sterile distilled water	qb 10	qb 10

Values are mg (ml for sterile distilled water)

TABLE III - CLINICAL EVALUATION

Parameter	Definition	Score
Corneal evaluation	Non re-epithelialized area	5
	Suture with epithelial lacune	4
	Suture with epithelial irregularities	3
	Well visible suture (>3 mm)	2
	Small suture (<3 mm)	1
	Disappearance of the fusion suture	0
Re-epithelialization	Non re-epithelialized area, calculated by multiplying the two transversal parameters (vertical and horizontal) of the lesion, expressed in mm	
Conjunctival hyperemia and palpebral edema	High	4
	Medium	3
	Light	2
	Absent	1
Pain	Pain intensity on a 10 cm Visual Analogue Scale	

standard home treatment envisaged the administration four times a day of two drops of single-dose diclofenac sodium 0.1% eye drops, tobramycin 0.3% eye drops, and netilmicin sulfate 0.3% eye drops.

The above mentioned procedure was used as an experimental model to study the effect of PDRN on the corneal re-epithelialization process. The patients enrolled in the study were randomly divided into two well-balanced groups. They were double-blindly treated either with PDRN eye drops or with placebo eye drops (consisting of only the excipients of the pharmacologically active eye drops) (two drops four times a day) together with standard therapy, starting from the day of surgery. Inclusion criteria were age over 21,obtained informed consent, and absence of eye or systemic diseases that could interfere with the re-epithelialization process. Exclusion criteria were participation in clinical studies still underway or within 2 months from enrollment and known PDRN hypersensitivity.

Patients were clinically examined immediately after surgery and at days 1, 2, 3, and 7 or until the lens removal, and the following parameters were evaluated: characteristics of corneal epithelialization, re-epithelialized area, conjunctival hyperemia, palpebral edema, and pain (Tab. III). Measurement of non re-epithelialized area was done using biomicroscopy (40 SL-P, Zeiss) and reading the width of the light beam exactly placed over the lesion, first horizontally and then vertically oriented. The width of the light beam was read on the control window of the slit-lamp with approximation to the first decimal and expressed in mm. Area was calculated by multiplying the two transversal parameters (vertical and horizontal) of the lesion and expressed in squared mm. All measurements were masked and performed by the same operator (M.L.).

The objective and subjective tolerability of the used drugs was evaluated at each control.

The study was approved by the Ethics Committee of the University of Siena and it was carried out in compliance with the Declaration of Helsinki and with good clinical practices.

Statistical analysis

Unless otherwise specified, the numerical values are indicated as a mean and standard deviation. The inferential analysis was carried out using the variance

analysis as a model. It has been followed by the Tukey-Kramer's multiple comparisons test and by the Student's t-test for the re-epithelialized corneal surface. As to the other data, the Fisher exact test, the Kruskal-Wallis analysis of variance by ranks followed by Dunn's multiple comparisons test were carried out. The minimum value for statistical significance was set at $p<0.05$.

RESULTS

On day 7, all the eyes were clinically healed; data analysis therefore refers to the course of the lesion between surgery and day 3. On day 3, 20 out of 26 (77%) corneas treated with PDRN and 17 out of 28 (61%) of those treated with a standard therapy were completely re-epithelialized (Fig.1). Such difference was not statistically significant with reference to absolute values. It was significant ($p<0.05$) if evaluated on percentages. On day 3, the analysis of the corneal evaluation score gave the following results: PDRN group mean score 2.9±1.4, placebo group mean score 3.8±1.0, $p<0.05$ (Tab. IV).

The disepithelialized surface at day 2, in the corneas treated with PDRN, was about 39.5% smaller if compared with the eyes treated with placebo (6.0±6.8 mm^2 vs 8.4±9.2) (not significant).

The Westlake 95% confidence limits (Fig. 2) confirmed the different healing trend between the groups during the first 2 days of observation. On day 3 the residual lesion surface, calculated on the not yet healed cases, looked similar in the two groups. After 2 days of observation, the different development of the repair process was in line with the different healing percentages of day 3. We therefore highlight that the value scatter is much lower in the patients treated with PDRN than in the patients treated with placebo: such data indicate that PDRN influences a uniform development of the repair process which on the contrary shows a more irregular course typical of spontaneous biological phenomena (Fig. 2) in controls.

In the five patients where one eye was treated with PDRN and the other with placebo (patients controls of themselves), on day 3, three out of five eyes treated with PDRN and two of those treated with placebo were healed. Table V shows the data concerning the re-epithelialization process.

The course found in all examined cases is confirmed (and more evident) in these subjects (Fig. 3). The data referring to conjunctival hyperemia, palpebral edema, and pain are summarized in Table VI. No clinically appreciable differences were found in the adopted therapeutic regimens. In no cases were adverse effects due to local or systemic drugs found.

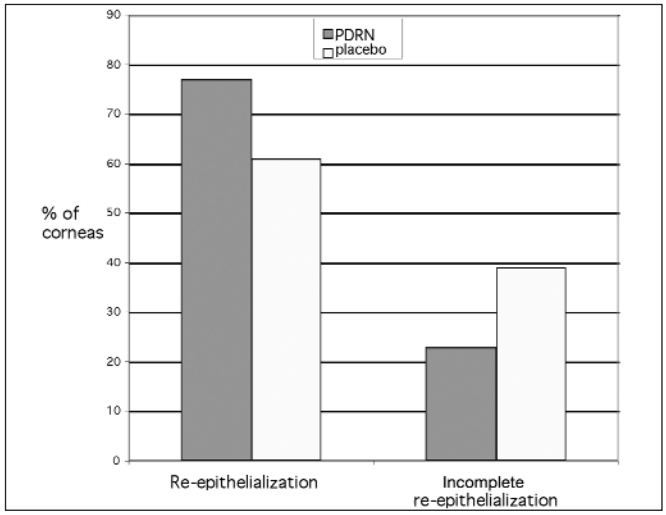

Fig. 1 - *Percentage of corneas with complete and incomplete re-epithelialization on day 3. PDRN = Polydeoxyribonucleotide.*

TABLE IV - CORNEAL EVALUATION SCORE (RANGE 1-5) (mean ± SD)

Eyes	Day 1	Day 2	Day 3
PDRN	5.0±0.0	5.0±0.0	2.9±1.4
Placebo	5.0±0.0	5.0±0.0	3.8±1.0

p<0.05 between groups in bold type
PDRN = Polydeoxyribonucleotide

DISCUSSION

In agreement with the literature, there is clinical evidence showing that a local or systemic administration of PDRN, in cases of tissue damage, is associated with activated organic repair processes in terms of stimulating healing and re-epithelialization. In fact, PDRN proved to be active and effective in the repair of lesions with loss of substance, in situations such as lower limb ulcers (18), anal fissures (19), skin explants (20), and burns (21).

The current clinical study evaluated the effect of PDRN

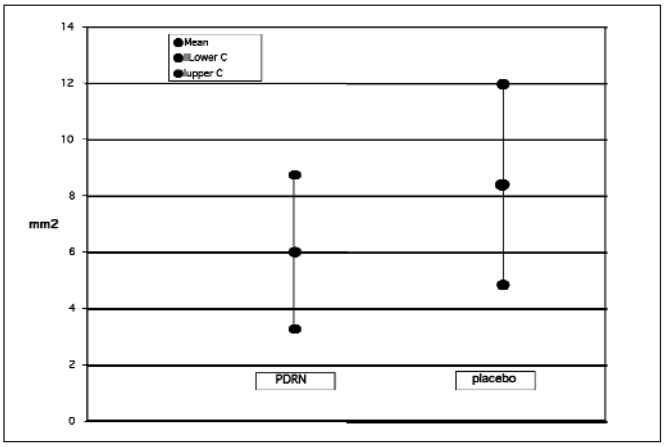

Fig. 2 - *Disepithelialized surface on day 2 (in mm²; mean values and relevant 95% confidence intervals). PDRN = Polydeoxyribonucleotide.*

TABLE V - SURFACE OF THE DISEPITHELIALIZED AREA IN THE CASES TREATED BOTH WITH PDRN AND PLACEBO (mm² mean ± SD)

Eyes	Day 1	Day 2	Day 3
PDRN (n=5)	32.9±20.1	4.7±2.8	0.5±0.6
Placebo (n=5)	34.1±13.0	10.8±8.0	1.7±1.6

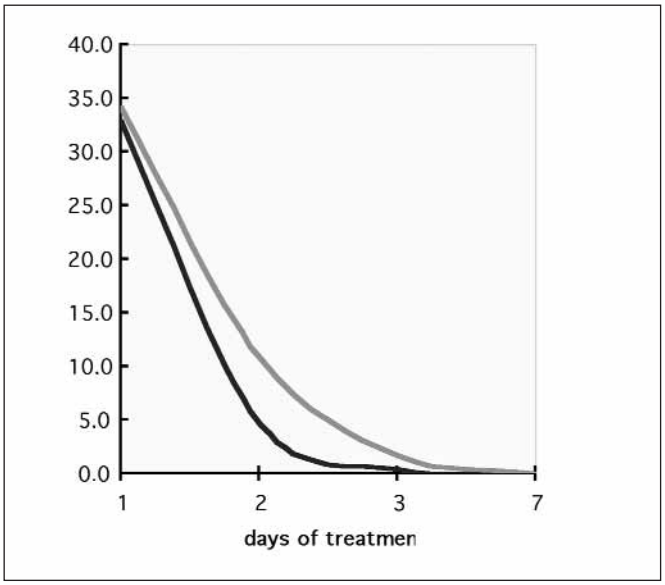

Fig. 3 - *Surface of the disepithelialized area in the cases treated both with polydeoxyribonucleotide (PRDN) and placebo.*

TABLE VI - CHANGES IN THE CLINICAL SYMPTOMS (mean ± SD)

Symptoms	Day 1	Day 2	Day 3
Conjunctival hyperemia (score 1>4)			
Eyes treated with PDRN	–	2.1±1.1	1.7±0.8
Eyes treated with placebo	–	2.6±0.8	1.7±0.7
Palpebral edema (score 1>4)			
Eyes treated with PDRN	–	2.1±1.1	1.7±0.9
Eyes treated with placebo	–	2.4±1.0	1.9±0.7
Pain (Visual Analogue Scale: mm)			
Eyes treated with PDRN	1.9±2.6	1.4±2.0	1.3±2.4
Eyes treated with placebo	2.6±2.7	1.8±2.5	0.5±1.2

PDRN = Polydeoxyribonucleotide

eye drops on the corneal re-epithelialization process in subjects who underwent PRK. An iatrogenic corneal lesion as an experimental model has the advantage of being standardized in terms of site, size, and nature. The variability typical of spontaneously developed lesions is therefore reduced. In addition, the opportunity of using in some cases a subject as his or her own control (in case of bilateral surgery) eliminates the variability linked to individual factors. Together with reproducible and consistent lesion features, there is a disadvantage due to the fact that in the adopted clinical conditions the corneal healing process is managed to obtain re-epithelialization as quickly as possible. This means that a more rapid repair is difficult to obtain. Therefore, under these circumstances, even small gains are important and reveal the drug's effect. The obtained data show that a greater re-epithelialization already occurs on day 2 in the treated corneas than in the controls. Furthermore, the evaluation on day 3 showed that 77% of the treated cases were re-epithelialized (20 out of 26) whereas 61% (17 out of 28) of the controls were re-epithelialized. Clinical evidence is shifted onto demonstrating the effect of the treatment, which is statistically significant ($p<0.05$) if evaluated in percentage, whereas it is not in terms of absolute values. On day 3, the analysis of the corneal evaluation score showed a statistically significant difference ($p<0.05$).

The collected data as well as those previously obtained in a pilot study (unpublished data) showed that PDRN eye drops associated with a local standard therapy based on anti-inflammatory drugs and antibiotics are able to shorten the corneal re-epithelialization process in subjects who underwent PRK.

Considering that advanced techniques adopted so far to reduce the re-epithelialization time provide little room for improvement, the data obtained in the study are in favor of a stimulating effect of PDRN on corneal re-epithelialization. Statistical significance was also obtained in some cases. A much larger clinical study should be performed in order to confirm the results obtained in this pilot study.

Reprint requests to:
Marco Lazzarotto, MD
Dipartimento di Scienze Oftalmologiche
e Neurochirurgiche Università di Siena
Ospedale Le Scotte
Viale Bracci
53100 Siena, Italy
m.lazzarotto@virgilio.it

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